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(54) Title: HYPOXIA–REGULATED GENES

(57) Abstract

According to the present invention polynucleotide sequences as set forth in SEQ ID No:1, SEQ ID No:2, SED ID No:3, SEQ ID No:4, SEQ ID No:5, SED ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11 are disclosed which are hypoxia regu lated. The present invention provides methods of regulating angiogenesis or apoptosis or regulating response to hypoxic conditions in a patient in need of such treatment. The present invention also provides a method of diagnosing the presence of ischemia in a patient including the steps of analyzing a bodily fluid or tissue sample from the patient for the presence or gene product of at least one expressed gene (up–regulated) as identified by the sequences of SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ No:11 and where ischemia is determined if the up–regulated gene or gene product is ascertained.

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HYPOXIA-REGULATED GENES

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

Identification of genes that are differentially expressed in hypoxia and use of the genes and gene products for diagnosis and therapeutic intervention.

2. DESCRIPTION OF RELATED ART

The level of tissue oxygenation plays an important role in normal development as well as in pathologic processes such as ischemia. Tissue oxygenation plays a significant regulatory role in both apoptosis and in angiogenesis (Bouck et al, 1996; Bunn et al, 1996; Dor et al, 1997; Carmeliet et al, 1998). Apoptosis (see Duke et al, 1996 for review) and growth arrest occur when cell growth and viability are reduced due to oxygen deprivation (hypoxia). Angiogenesis (i.e. blood vessel growth, vascularization), is stimulated when hypooxygenated cells secrete factors which stimulate proliferation and migration of endothelial cells in an attempt to restore oxygen homeostasis (for review see Hanahan et al, 1996).

Ischemic disease pathologies involve a decrease in the blood supply to a bodily organ, tissue or body part generally caused by constriction or obstruction of the blood vessels as for example retinopathy, acute renal failure, myocardial infarction and stroke. Therefore apoptosis and angiogenesis as induced by the ischemic condition are also involved in these disease states. Neoangiogenesis is seen in some forms of retinopathy and in tumor growth. It is recognized that angiogenesis is necessary for tumor growth and that retardation of angiogenesis would be a useful tool in controlling malignancy and retinopathies. Further, it would be useful to induce tumorigenic cells to undergo apoptosis (i.e. programmed cell death).

However, these processes are complex cascades of events controlled by many different genes reacting to the various stresses such as hypoxia. Expression of different genes reacting to the hypoxic stress can trigger not only apoptosis or angiogenesis but both. In cancer it has been observed that apoptosis and angiogenesis related genes are therapeutic targets. However, hypoxia itself plays a critical role in the selection of mutations that contribute to more severe tumorigenic phenotypes (Graeber et al., 1996). Therefore identifying candidate genes and gene products that can be utilized therapeutically not only in cancer and ischemia

and that may either induce apoptosis or angiogenesis or to retard the processes is needed. It would be useful to identify genes that have direct causal relationships between a disease and its related pathologies and an up- or down-regulator (responder) gene.

SUMMARY OF THE INVENTION

According to the present invention, purified, isolated and cloned polynucleotides (alternatively referred to as nucleic acid sequences) having hypoxia regulated activity are identified by the sequences as set forth in SEQ ID Nos:1-11 or having a complementary or allelic variation sequence thereto or human homolog thereof are disclosed.

The present invention provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein as encoded by the gene whose nucleic acid sequences are identified by the sequences as set forth in SEQ ID Nos:1-11 or by administering the protein itself. Alternatively, the present invention provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least

one antisense oligonucleotide directed against the nucleic acid sequences as set forth in SEQ ID Nos:1-11 or dominant negative peptide or cDNA directed against the nucleic acid or protein encoded by the seqeunces.

The present invention provides a method of providing an apoptotic regulating gene by administering directly to a patient in need of such therapy, utilizing gene therapy, an expressible vector comprising expression control sequences operably linked to one of the gene which is identified by the sequences set forth in SEQ ID Nos:1-11 or the human homolog as appropriate.

The present invention also provides a method of providing an angiogenesis regulating gene utilizing gene therapy by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to a gene identified by one of the sequences set forth in SEQ ID Nos:1-11 or the human homolog as appropriate.

The present invention provides a method of regulating response to hypoxic conditions in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in SEQ ID Nos:1-11. The present invention further provides a method of providing a hypoxia regulating gene utilizing gene therapy by

administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to the gene identified by one of the sequences set forth in SEQ ID Nos:1-11 or the human homolog as appropriate.

The present invention also provides a method of diagnosing the presence of ischemia in a patient including the steps of analyzing a bodily fluid or tissue sample from the patient for the presence or gene product of at least one expressed gene (up-regulated) as identified by the sequences set forth in SEQ ID Nos:1-11 and where ischemia is determined if the up-regulated gene or gene product is ascertained.

DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIGURE 1 is a computer scan showing RTP569 (SEQ ID No:10) Alternative splicing factor SF2p33 Northern blot analysis. RNA was extracted from Rat C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours.

PolyA+ selected mRNA (2ug) from each sample were separated on denaturing agarose gels, bloted onto Nytran membranes and hybridized with rtp569 probe. The reduction in mRNA levels, as predicted from the DNA chip analysis is clearly observed.

FIGURE 2 is a computer scan showing RTP920 (SEQ ID No:11) Ring zinc finger Northern blot analysis. RNA was extracted from Rat C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours. PolyA+ selected mRNA (2ug) from each sample were separated on denaturing agarose gels, bloted onto Nytran membranes and hybridized with rtp920 probe.

FIGURE 3 is a computer scan showing RTP751 (SEQ ID No:9) Lysyl hydroxilase Northern blot analysis. RNA was extracted from Rat C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours. PolyA+ selected mRNA (2ug) from each sample were separated on denaturing agarose gels, blotted onto Nytran membranes and hybridized with rtp751 probe. One band of 3.5Kb is observed showing extreme differential expression.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies candidate genes and gene products that can be utilized therapeutically and diagnostically in hypoxia and ischemia and that may regulate apoptosis or angiogenesis. By regulate or modulate or control is meant that the process is either induced or inhibited to the degree necessary to effect a change in the process and the associated disease state in the patient. Whether induction or inhibition is being contemplated will be apparent from the process and disease being treated and will be known to those skilled in the medical arts. The present invention identifies genes for gene therapy, diagnostic and therapeutics that have direct causal relationships between a disease and its related pathologies and up- or down-regulator (responder) genes. That is the present invention is initiated by a physiological relationship between cause and effect.

The present invention identifies hypoxia-regulated nucleic acid sequences which identify genes which respond at least to hypoxic conditions by up-regulation of expression and which have sequences as set forth in SEQ ID Nos:1-11 and their analogues and polymorphisms or a complementary or allelic variation sequence thereto or human homologs as appropriate.

The present invention further provides proteins and their analogues as encoded by the genes identified by the nucleic acid sequences as set forth in SEQ ID Nos:1-11 for use as therapeutics and diagnositcs in hypoxia associated pathologies such as ischemia. The proteins may be produced recombinantly (see generally Marshak et al, 1996 "Strategies for Protein Purification and Characterization. A laboratory course manual." CSHL Press) and analogues may be due to post-translational processing. The term Analogue as used herein is defined as a nucleic acid sequence or protein which has some differences in their amino acid/nucleotid sequences as compared to the native sequence of SEQ ID Nos:1-11. Ordinarily, the analogue will be generally at least 70% homologous over any portion that is functionally relevant. In more preferred embodiments the homology will be at least 80% and can approach 95% homology to the protein/nucleotide sequence. The amino acid or nucleotide sequence of an analog may differ from that of the primary sequence when at least one residue is deleted, inserted or substituted, but the protein or nucleic acid molecule remains functional. Differences in glycosylation can provide protein analogues.

Functionally relevant refers to the biological property of the molecule and in this context means an *in vivo* effector or antigenic function or activity that is

directly or indirectly performed by a naturally occurring protein or nucleic acid molecule. Effector functions include but are not limited to include receptor binding, any enzymatic activity or enzyme modulatory activity, any carrier binding activity, any hormomal activity, any activity in promoting or inhibiting adhesion of cells to extracellulat matrix or cell surface molecules, or any structural role as well as having the nucleic acid sequence encode functional protein and be expressible. The antigenic functions essentially mean the possession of an epitope or antigenic site that is capable of cross-reating with antiboides raised against a naturally occurring protein. Biologically active analogues share an effector function of the native which may, but need not, in addition possess an antigenic function.

The present invention utilizes antibodies directed against the proteins as encoded by the nucleic acid sequences as set forth in SEQ ID Nos:1-11 which can be used in immunoassays and the like as part of the diagnostic procedures for identifying ischemic conditions.

The antibodies may be either monoclonal, polyclonal or recombinant. Conveniently, the antibodies may be prepared against the immunogen or portion thereof for example a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the

natural gene product and/or portions thereof may be isolated and used as the immunogen. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art as described generally in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992. Antibody fragments may also be prepared from the antibodies and include Fab, $F(ab')_2$, and Fv by methods known to those skilled in the art.

For producing polyclonal antibodies a host, such as a rabbit or goat, is immunized with the immunogen or immunogen fragment, generally with an adjuvant and, if necessary, coupled to a carrier; antibodies to the immunogen are collected from the sera. Further, the polyclonal antibody can be absorbed such that it is monospecific. That is, the sera can be absorbed against related immunogens so that no cross-reactive antibodies remain in the sera rendering it monospecific.

For producing monoclonal antibodies the technique involves hyperimmunization of an appropriate donor with the immunogen, generally a mouse, and isolation of splenic antibody producing cells. These cells are fused to a cell having immortality, such as a myeloma cell, to provide a fused cell hybrid which has immortality and

secretes the required antibody. The cells are then cultured, in bulk, and the monoclonal antibodies harvested from the culture media for use.

For producing recombinant antibody (see generally Huston et al, 1991; Johnson and Bird, 1991; Mernaugh and Mernaugh, 1995), messenger RNAs from antibody producing B-lymphocytes of animals, or hybridoma are reverse-transcribed to obtain complimentary DNAs (cDNAs). Antibody cDNA, which can be full or partial length, is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. (For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the art. (see for a general discussion Harlow & Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor

Laboratory Publications, New York, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992) The detectable moieties contemplated with the present invention can include, but are not limited to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, β-galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, ^{14}C and iodination.

The present invention provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein as encoded by the gene identified by the nucleic acid sequences as set forth in SEQ ID Nos:1-11. The antagonist is dosed and delivered as an active ingredient in a pharmaceutically acceptable carrier as described herein below. The term antagonist or antagonizing is used in its broadest sense. Antagonism can include any mechanism or treatment which results in inhibition, inactivation, blocking or reduction in gene activity or gene product. It should be noted that the inhibition of a gene or gene product may provide for an increase in a corresponding function that the gene or gene product was regulating. The antagonizing step can include blocking cellular receptors for the gene product of SEQ ID Nos:1-11 and can include antisense treatment as

discussed herein below. For example, a patient may be in need of inducing apoptosis in tumorigenic cells or angiogenesis in trauma situations where for example a limb must be reattached or in a transplant where revascularization is needed.

The present invention provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one antisense oligonucleotide or dominant negative peptide (either as cDNA or peptide; Herskowitz, 1987) directed against at least one of the nucleic acid sequences as set forth in SEQ ID Nos:1-11. The present invention also provides a method of regulating response to hypoxic conditions in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in SEQ ID Nos:1-11. The antisense oligonucleotide as active ingredient in a pharmaceutical composition is dosed and delivered in a pharmaceutically acceptable carrier as discussed herein below.

Many reviews have covered the main aspects of antisense (AS) technology and its enormous therapeutic potential (Wright and Anazodo, 1995). There are reviews on the chemical (Crooke, 1995; Uhlmann et al, 1990),

cellular (Wagner, 1994) and therapeutic (Hanania, *et al*, 1995; Scanlon, *et al*, 1995; Gewirtz, 1993) aspects of this rapidly developing technology. Within a relatively short time, ample information has accumulated about the *in vitro* use of AS nucleotide sequences in cultured primary cells and cell lines as well as for *in vivo* administration of such nucleotide sequences for suppressing specific processes and changing body functions in a transient manner. Further, enough experience is now available *in vitro* and *in vivo* in animal models and human clinical trials to predict human efficacy.

Antisense intervention in the expression of specific genes can be achieved by the use of synthetic AS oligonucleotide sequences (for recent reports see Lefebvre-d'Hellencourt *et al*, 1995; Agrawal, 1996; Lev-Lehman *et al*, 1997). AS oligonucleotide sequences may be short sequences of DNA, typically 15-30 mer but may be as small as 7 mer (Wagner et al, 1996), designed to complement a target mRNA of interest and form an RNA:AS duplex. This duplex formation can prevent processing, splicing, transport or translation of the relevant mRNA. Moreover, certain AS nucleotide sequences can elicit cellular RNase H activity when hybridized with their target mRNA, resulting in mRNA degradation (Calabretta *et al*, 1996). In that case, RNase H will cleave the RNA

component of the duplex and can potentially release the AS to further hybridize with additional molecules of the target RNA. An additional mode of action results from the interaction of AS with genomic DNA to form a triple helix which may be transcriptionally inactive.

The sequence target segment for the antisense oligonucleotide is selected such that the sequence exhibits suitable energy related characteristics important for oligonucleotide duplex formation with their complementary templates, and shows a low potential for self-dimerization or self-complementation [Anazodo et al., 1996]. For example, the computer program OLIGO (Primer Analysis Software, Version 3.4), can be used to determine antisense sequence melting temperature, free energy properties, and to estimate potential self-dimer formation and self-complimentary properties. The program allows the determination of a qualitative estimation of these two parameters (potential self-dimer formation and self-complimentary) and provides an indication of "no potential" or "some potential" or "essentially complete potential". Using this program target segments are generally selected that have estimates of no potential in these parameters. However, segments can be used that have "some potential" in one of the categories. A balance of the parameters is used in the selection as is known in the art. Further, the oligonucleotides are also

selected as needed so that analogue substitution do not substantially affect function.

Phosphorothioate antisense oligonucleotides do not normally show significant toxicity at concentrations that are effective and exhibit sufficient pharmacodynamic half-lives in animals (Agarwal *et al.*, 1996) and are nuclease resistant. Antisense induced loss-of-function phenotypes related with cellular development were shown for the glial fibrillary acidic protein (GFAP), for the establishment of tectal plate formation in chick (Galileo *et al.*, 1991) and for the N-myc protein, responsible for the maintenance of cellular heterogeneity in neuroectodermal cultures (ephithelial vs. neuroblastic cells, which differ in their colony forming abilities, tumorigenicity and adherence) (Rosolen *et al.*, 1990; Whitesell *et al*, 1991). Antisense oligonucleotide inhibition of basic fibroblast growth factor (bFgF), having mitogenic and angiogenic properties, suppressed 80% of growth in glioma cells (Morrison, 1991) in a saturable and specific manner. Being hydrophobic, antisense oligonucleotides interact well with phospholipid membranes (Akhter *et al.*, 1991). Following their interaction with the cellular plasma membrane, they are actively (or passively) transported into living cells (Loke *et al.*, 1989), in a saturable mechanism predicted to involve specific receptors (Yakubov *et al.*, 1989).

Instead of an antisense sequence as discussed herein above, ribozymes may be utilized. This is particularly necessary in cases where antisense therapy is limited by stoichiometric considerations (Sarver et al., 1990, Gene Regulation and Aids, pp. 305-325). Ribozymes can then be used that will target the same sequence. Ribozymes are RNA molecules that possess RNA catalytic ability (see Cech for review) that cleave a specific site in a target RNA. The number of RNA molecules that are cleaved by a ribozyme is greater than the number predicted by stochiochemistry. (Hampel and Tritz, 1989; Uhlenbeck, 1987).

Ribozymes catalyze the phosphodiester bond cleavage of RNA. Several ribozyme structural families have been identified including Group I introns, RNase P, the hepatitis delta virus ribozyme, hammerhead ribozymes and the hairpin ribozyme originally derived from the negative strand of the tobacco ringspot virus satellite RNA (sTRSV) (Sullivan, 1994; U.S. Patent No. 5,225,347, columns 4-5). The latter two families are derived from viroids and virusoids, in which the ribozyme is believed to separate monomers from oligomers created during rolling circle replication (Symons, 1989 and 1992). Hammerhead and hairpin ribozyme motifs are most commonly adapted for trans-cleavage of mRNAs for gene therapy (Sullivan, 1994). The ribozyme type utilized in the

present invention is selected as is known in the art. Hairpin ribozymes are now in clinical trial and are the preferred type. In general the ribozyme is from 30-100 nucleotides in length.

Modifications or analogues of nucleotides can be introduced to improve the therapeutic properties of the nucleotides. Improved properties include increased nuclease resistance and/or increased ability to permeate cell membranes.

Nuclease resistance, where needed, is provided by any method known in the art that does not interfere with biological activity of the antisense oligodeoxy-nucleotides, cDNA and/or ribozymes as needed for the method of use and delivery (Iyer et al., 1990; Eckstein, 1985; Spitzer and Eckstein, 1988; Woolf et al., 1990; Shaw et al., 1991). Modifications that can be made to oligonucleotides in order to enhance nuclease resistance include modifying the phophorous or oxygen heteroatom in the phosphate backbone. These include preparing methyl phosphonates, phosphorothioates, phosphorodithioates and morpholino oligomers. In one embodiment it is provided by having phosphorothioate bonds linking between the four to six 3'-terminus nucleotide bases. Alternatively, phosphorothioate bonds link all the nucleotide bases. Other modifications known in the art may be used where

the biological activity is retained, but the stability to nucleases is substantially increased.

The present invention also includes all analogues of, or modifications to, an oligonucleotide of the invention that does not substantially affect the function of the oligonucleotide. The nucleotides can be selected from naturally occurring or synthetic modified bases. Naturally occurring bases include adenine, guanine, cytosine, thymine and uracil. Modified bases of the oligonucleotides include xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza cytosine and 6-aza thymine, psuedo uracil, 4-thiuracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other substituted guanines, other aza and deaza adenines, other aza and deaza guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

In addition, analogues of nucleotides can be prepared wherein the structure of the nucleotide is fundamentally altered and that are better suited as therapeutic or experimental reagents. An example of a nucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in

DNA (or RNA0 is replaced with a polyamide backbone which is similar to that found in peptides. PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. Further, PNAs have been shown to bind stronger to a complementary DNA sequence than a DNA molecule. This observation is attributed to the lack of charge repulsion between the PNA strand and the DNA strand. Other modifications that can be made to oligonucleotides include polymer backbones, cyclic backbones, or acyclic backbones.

The active ingredients of the pharmaceutical composition can include oligonucleotides that are nuclease resistant needed for the practice of the invention or a fragment thereof shown to have the same effect targeted against the appropriate sequence(s) and/or ribozymes. Combinations of active ingredients as disclosed in the present invention can be used including combinations of antisense sequences.

The antisense oligonucleotides (and/or ribozymes) and cDNA of the present invention can be synthesized by any method known in the art for ribonucleic or deoxyribonucleic nucleotides. For example, an Applied Biosystems 380B DNA synthesizer can be used. When fragments are used, two or more such sequences can be synthesized and linked together for use in the present invention.

The nucleotide sequences of the present invention can be delivered either directly or with viral or non-viral vectors. When delivered directly the sequences are generally rendered nuclease resistant. Alternatively the sequences can be incorporated into expression cassettes or constructs such that the sequence is expressed in the cell as discussed herein below. Generally the construct contains the proper regulatory sequence or promotor to allow the sequence to be expressed in the targeted cell.

Negative dominant peptide refers to a partial cDNA sequence that encodes for a part of a protein, i.e. a peptide (see Herskowitz, 1987). This peptide can have a different function from the protein it was derived from. It can interact with the full protein and inhibit its activity or it can interact with other proteins and inhibit their activity in response to the full protein. Negative dominant means that the peptide is able to overcome the natural proteins and fully inhibit their activity to give the cell a different characteristics like resistance or sensitization to killing. For therapeutic intervention either the peptide itself is delivered as the active ingredient of a pharmaceutical composition or the cDNA can be delivered to the cell utilizing the same methods as for antisense delivery.

The present invention provides a method of providing an apoptotic regulating gene, angiogenesis regulating

gene or a hypoxia regulating gene by administering directly to a patient in need of such therapy utilizing gene therapy an expressible vector comprising expression control sequences operably linked to a gene identified by one of the sequences set forth in SEQ ID Nos:1-11 or the human homolog as appropriate.

By gene therapy as used herein refers to the transfer of genetic material (e.g DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The geneitc material is generally species specific or has been modified to be species specific. For example where the sequence has been identified in one specied the counterpart, homolog, of the transfer receipient is used as necessary if there is not a high enough level of homology between the two species. The genetic material of interest encodes a product (e.g. a protein, polypeptide, peptide, functional RNA, antisense) whose production in vivo is desired. For example, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic value. Alternatively, the genetic material of interest encodes a suicide gene. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo*

gene therapy cells are removed from a patient, and while being cultured are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to express the transfected genetic material *in situ*.

In *in vivo* gene therapy, target cells are not removed from the subject rather the genetic material to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. In an alternative embodiment, if the host gene is defective, the gene is repaired *in situ* [Culver, 1998]. These genetically altered cells have been shown to express the transfected genetic material *in situ*.

The gene expression vehicle is capable of delivery/transfer of heterologous nucleic acid into a host cell. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle. Therefore as used

herein the expression vehicle may, as needed, not include the 5'UTR and/or 3'UTR of the actural gene to be transferred and only include the specific amino acid coding region.

The expression vehicle can include a promotor for controlling transcription of the heterologous material and can be either a constitutive or inducible promotor to allow selective transcription. Enhancers that may be required to obtain necessary transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. The expression vehicle can also include a selection gene as described herein below.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988)

and Gilboa et al (1986) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see United States patent 4,866,042 for vectors involving the central nervous system and also United States patents 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of DNA viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that

exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur.

Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in

the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the invention will depend on desired cell type to be targeted and will be known to those skilled in the art. For example, if breast cancer is to be treated then a vector specific for such epithelial cells would be used. Likewise, if diseases or pathological conditions of the hematopoietic system are to be treated, then a viral

vector that is specific for blood cells and their precursors, preferably for the specific type of hematopoietic cell, would be used.

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains all the necessary genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new viral particles which are capable of undergoing further rounds of infection. The vector's genome is also engineered to encode and express the desired recombinant gene. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles. Without such a signal, any particles that are formed will not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector will depend upon the intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

The recomnbinant vector can be administered in several ways. If viral vectors are used, for example,

the procedure can take advantage of their target specificity and consequently, do not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Injection of the viral vectors into a spinal fluid can also be used as a mode of administration, especially in the case of neuro-degenerative diseases. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients or into the spinal fluid. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for

the desired subset can be used to accomplish this goal. Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

The pharmaceutical compositions of the present invention containing the active ingredients of the present invention as decribed herein above are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the medical arts. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the medical arts. The pharmaceutical compositions can be combinations of the active

ingrediants but will include at least one active ingredient.

In the method of the present invention, the pharmaceutical compositions of the present invention can be administered in various ways taking into account the nature of compounds, active ingredients, in the pharmaceutical compositions. It should be noted that they can be administered as the compound or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein which treatment has a length proportional to the

length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

The doses may be single doses or multiple doses over a period of several days. The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated.

When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent

systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present

invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include: 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques which deliver it orally or intravenously and retain the biological activity are preferred.

In one embodiment, the compound of the present invention can be administered initially by intravenous injection to bring blood levels to a suitable level. The patient's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity to be administered will vary for

the patient being treated and will vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably will be from 10 μg/kg to 10 mg/kg per day.

The present invention also provides a method of diagnosing the presence of ischemia in a patient including the steps of analyzing a bodily fluid or tissue sample from the patient for the presence or gene product of at least one expressed gene (up-regulated) as identified by the sequences as set forth in SEQ ID Nos:1-11 or their gene product and where ischemia is determined if the up-regulated gene or gene product is ascertained as described herein in the Example. The bodily fluids may include tears, serum, urine, sweat or other bodily fluid where secreted proteins from the tissue that is undergoing an ischemic event may be localized. Additional methods for identification of the gene or gene product are immunoassays, such as and ELISA or radioimmunoassays (RIA), can be used as are known to those in the art particularly to identify gene products in the samples. Immunohistochemical staining of tissue samples is also utilized for identification. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219;

5,011,771 and 5,281,521. Further for identification of the gene, *in situ* hybridization, Southern blotting, single strand conformational polymorphism, restriction endonuclease fingerprinting (REF), PCR amplification and DNA-chip analysis using nucleic acid sequence of the present invention as primers can be used.

The above discussion provides a factual basis for the use of genes to regulate hypoxia and ischemia and thereby also apoptosis and angiogenesis. The methods used with and the utility of the present invention can be shown by the following non-limiting example and accompanying figures.

EXAMPLE

METHODS:

Most of the techniques used in molecular biology are widely practiced in the art, and most practitioners are familiar with the standard resource materials which describe specific conditions and procedures. However, for convenience, the following paragraphs may serve as a guideline.

General methods in molecular biology: Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989), and in

Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989) particularly for the Northern Analysis and In Situ analysis and in Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988), and in Watson et al., *Recombinant DNA*, Scientific American Books, New York. Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990).

Reactions and manipulations involving other nucleic acid techniques, unless stated otherwise, were performed as generally described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, and methodology as set forth in United States patents 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057 and incorporated herein by reference.

Additionally, In situ (In cell) PCR in combination with flow cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni et al, 1996, Blood 87:3822).

General methods in immunology: Standard methods in immunology known in the art and not specifically described are generally followed as in Stites et al.(eds), Basic and Clinical Immunology (8th Edition),

Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), Selected Methods in Cellular Immunology, W.H. Freeman and Co., New York (1980). Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989.

Differential Analysis

For example C6 glioma cells or other appropriate cells, cell lines or tissues are grown under normal conditions (Normoxia) or under oxygen deprivation conditions (Hypoxia) generally for four to sixteen hours. The cells are harvested and RNA is prepared from the cytoplasmic extracts and from the nuclear fractions. Following the extraction of RNA, fluorescent cDNA probes are prepared. Each condition (for example 4 hours hypoxia and normoxia) is labeled with a different fluorescent dye. For example a probe can be composed of a mixture of Cy3 -dCTP cDNA prepared from RNA extracted from hypoxic cells and with Cy5-dCTP cDNA prepared from RNA extracted from normoxic cells. The probes are used for hybridization to micro-array containing individually

spotted cDNA clones derived from C6 cells that were exposed to hypoxia. Differential expression in measured by the amount of fluorescent cDNA that hybridizes to each of the clones on the array. Genes that are up regulated under hypoxia will have more fluorescence of Cy3 than Cy5. The results show genes that are transcriptionally induced mRNA species that respond very fast to hypoxia.

Differential display:

Reverse transcription: 2μg of RNA are annealed with 1pmol of oligo dT primer $(dT)_{18}$ in a volume of 6.5μl by heating to 70°C for five minutes and cooling on ice. 2μl reaction buffer (x5), 1μl of 10mM dNTP mix, and 0.5μl of SuperScript II reverse transcriptase (GibcoBRL) is added. The reaction is carried for one hour at 42°C. The reaction is stopped by adding 70μl TE (10mM Tris pH=8; 0.1mM EDTA).

Oligonucleotides used for Differential display: The oligonucleotides are generally those described in the Delta RNA Fingerprinting kit (Clonetech Labs. Inc.).

Amplification reactions: Each reaction is done in 20μl and contains 50μM dNTP mix, 1μM from each primer, 1x polymerase buffer, 1 unit expand Polymerase (Beohringer Mannheim), 2μCi [α-^{32}P]dATP and 1μl cDNA template. Cycling conditions are generally: three minutes at 95°C, then three cycles of two minutes at 94°C, five minutes at 40°C, five minutes at 68°C. This is followed by 27 cycles

of one minute at 94°C, two minutes at 60°C, two minutes at 68°C. Reactions were terminated by a seven minute incubation at 68°C and addition of 20μl sequencing stop solution (95% formamide, 10mM NaOH, 0.025% bromophenol blue, 0.025% xylene cyanol).

Gel analysis: Generally 3-4μl are loaded onto a 5% sequencing polyacrylamide gel and samples are electrophoresed at 2000 volts/40 milliamperes until the slow dye (xylene cyanol) is about 2 cm from the bottom. The gel is transferred to a filter paper, dryed under vacuum and exposed to x-ray film.

Recovery of differential bands: Bands showing any a differential between the various pools are excised out of the dried gel and placed in a microcentrifuge tube. 50μl of sterile H_2O are added and the tubes heated to 100°c for five minutes. 1μl is added to a 49μl PCR reaction using the same primers used for the differential display and the samples are amplified for 30 cycles of: one minute at 94°C, one minute at 60°C and one minute at 68°C. 10μl is analyzed on agarous gel to visualize and confirm successful amplification.

Representational difference analysis

Reverse transcription: as above but with 2μg polyA+ selected mRNA.

Preparation of double stranded cDNA: cDNA from the previous step is treated with alkali to remove the mRNA,

precipitated and dissolved in 20μl H_2O. 5μl buffer, 2μl 10mM dATP, H_2O to 48μl and 2μl terminal deoxynucleotide transferase (TdT) are added. The reaction is incubated 2-4 hours at 37°C. 5μl oligo dT (1μg/μl) was added and incubated at 60°C for five minutes. 5μl 200 mM DTT, 10 μl 10x section buffer (100mM Mg Cl_2, 900 mM Hepes, pH 6.6) 16 μl dNTPs (1 mM), and 16 U of Klenow are added and the mixture incubated overnight at room temperature to generate ds cDNA. 100μl TE is added and extracted with phenol/chloroform. The DNA is precipitated and dissolved in 50μl H_2O.

Generation of representations: cDNA with DpnII is digested by adding 3μl DpnII reaction buffer 20 V and DpnII to 25μl cDNA and incubated five hours at 37°C. 50μl TE is added and extracted with phenol/chloroform. cDNA is precipitated and dissolved to a concentration of 10ng/μl.

Driver: 1.2μg DpnII digested cDNA. 4μl from each oligo and 5μl ligation buffer x10 and annealed at 60°C for ten minutes. 2μl ligase is added and incubated overnight at 16°C. The ligation mixture is diluted by adding 140μl TE. Amplification is carried out in a volume of 200μl using appropriate primer and 2μl ligation product and repeated in twenty tubes for each sample. Before adding Taq DNA polymerase, the tubes are heated to 72°C for three minutes. PCR conditions are as follows: five minutes at

72°C, twenty cycles of one minute at 95°C and three minutes at 72°C, followed by ten minutes at 72°C. Every four reactions were combined, extracted with phenol/chloroform and precipitated. Amplified DNA is dissolved to a concentration of 0.5μg/μl and all samples are pooled.

Subtraction: Tester DNA (20μg) is digested with DpnII as above and separated on a 1.2% agarous gel. The DNA is extracted from the gel and 2μg ligated to the apprpriate oligos. The ligated Tester DNA is then diluted to 10ng/μl with TE. Driver DNA is digested with DpnII and repurified to a final concentration of 0.5μg/μl. Mix 40μg of Driver DNA with 0.4μg of Tester DNA. Extraction is carried out with phenol/chloroform and precipitated using two washs with 70% ethanol, resuspended DNA in 4μl of 30mM EPPS pH=8.0, 3mM EDTA and overlayed with 35μl mineral oil. Denature at 98°C for five minutes, cool to 67°C and 1μl of 5M NaCl added to the DNA. Incubate at 67°C for twenty hours. Dilute DNA by adding 400μl TE.

Amplification: Amplification of subtracted DNA in a final volume of 200μl as follows: Buffer, nucleotides and 20μl of the diluted DNA are added, heated to 72°C, and Taq DNA polymerase added. Incubate at 72°C for five minutes and add apprpriate oligo. Ten cycles of one minute at 95°C, three minutes at 70°C are performed. Incubate ten minutes at 72°C. The amplification is

repeated in four separate tubes. The amplified DNA is extracted with phenol/chloroform, precipitated and all four tubes combined in 40μl 0.2xTE, and digested with Mung Bean Nuclease as follows: To 20μl DNA 4μl buffer, 14μl H_2O and 2μl Mung Bean Nuclease (10 units/μl) added. Incubate at 30°C for thirty-five minutes + First Differential Product (DPI).

Repeat subtraction hybridization and PCR amplification at driver: differential ratio of 1:400 (DPII) and 1:40,000 (DPIII) using apprpriate oligonucleotides. Differential products are then cloned into a Bluescript vector at the BAM HI site for analysis of the individual clones.

DIFFERENTIAL EXPRESSION USING GENE EXPRESSION MICRO-ARRAY

Messanger RNA isolated as described herein above is labeled with fluorescent dNTP's using a reverse transcription reaction to generate a labeled cDNA probe. mRNA is extracted from C6 cells cultured in normoxia conditions and labeled with Cy3-dCTP (Amersham) and mRNA extracted from C6 cells cultured under hypoxia conditions is labeled with Cy5-dCTP (Amersham). The two labeled cDNA probes are then mixed and hybridized onto a microarray (Schena et al, 1996) composed of for example 2000 cDNA clones derived from a cDNA library prepared from C6 cells cultured under hypoxic conditions. Following hybridizaition the microarray is scanned using

a laser scanner and amount of fluoescence of each of the fluorescence dyes is measured for each cDNA clone on the micro-array giving an indication of the level of mRNA in each of the original mRNA populations being tested. Comparison of the fluorescence on each cDNA clone on the micro-array between the two differenct fluorescent dyes is a measure for the differential expression of the indicated genes between the two experimental conditions.

IN SITU ANALYSIS

In situ analysis is performed for the candidate genes identified by the differential response to exposure to hypoxia conditions as described above. The expression is studied in two experimental systems: solid tumors and hypoxic retina.

Solid tumors are formed by injections in mice of the original glioma cells used for the differential expression. The glioma cells form tumors which are then excised, slided and used to individually measure expression levels of the candidate gene. The solid tumor model (Benjamin et alm, 1997) shows that the candidate gene's expression is activated in tumors around the hypoxic regions that are found in the center of the tumor and are therefore hypoxia-regulated *in vivo*. Up regulation indicates further that the up-regulated gene

can promote angiognesis that is required to sustain tumor growth.

The hypoxia retina model measures expression levels in an organ that is exposed to hypoxia (ischemia) and directly mimics retinopathy. Hypoxia in the retina is created by exposing new born rats to hyperoxia which diminishes blood vessels in the retinas (Alon et al., 1995). Upon transfer to normal oxygen levels, relative hypoxia is formed due to the lack of blood supply. The hypoxic retina is excised, sliced and used to monitor the expression of the candidate genes.

RESULTS

Utilizing gene expression microarray analysis the sequences set forth in SEQ ID Nos:1-11 were identified as being differentially expressed under hypoxia conditions and as shown in the figures differential expression under hypoxia conditions was observed. Northern Analysis was performed with 32P-dCTP labeled probes derived from the candidate genes. Two micrograms of mRNA were fractionated on formaldehyde containing agarose gels, blotted onto a nitrocellulose membrane and hybridized to the labeled cDNA probes. To monitor the kinetics of expression as a result of hypoxia, mRNA was prepared from cells in normoxia, and 4 and 16 hours exposure to hypoxia conditions. The results of the analysis confirmed that

the results obtained by the gene expression microarray analysis can be used to determine hypoxia-regulated repsonse. The following are the sequences and the identification of the gene that was found to be a match in the data base search.

SEQ ID No:1 (RTP200)	Connective tissue growth factor FISP-12
SEQ ID No:2 (RTP269)	Putative zinc finger protein - involved in hypoxia specific transcriptional activation.
SEQ ID No:3 (RTP483)	Ring 3 human- involved in hypoxia specific transcriptional activation
SEQ ID No:4 (RTP599)	Lon protease-like (ATP-dependent)
SEQ ID No:5 (RTP633)	Serin protease with IGF binding motif
SEQ ID No:6 (RTP651)	Neutral amino acid transporter
SEQ ID No:7 (RTP883)	Serin proease, human
SEQ ID No:8 (RTP291)	CE9 transmembrane glycoprotein
SEQ ID No:9 (RTP751)	Lysyl hydroxylase
SEQ ID No:10 (RTP569)	Alternative splicing factor, human SF2p33
SEQ ID No:11 (RTP920)	Ring zinc finger, chicken

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number.

Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Einat, Paz
Skaliter, Rami

(ii) TITLE OF INVENTION: HYPOXIA-REGULATED GENES

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 30500 Northwestern Hwy., Suite 410
(C) CITY: Farminton Hills
(D) STATE: Michigan
(E) COUNTRY: U.S.
(F) ZIP: 48334

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kohn, Kenneth I.
(B) REGISTRATION NUMBER: 30,955
(C) REFERENCE/DOCKET NUMBER: 0168.00039

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (248) 539-5050
(B) TELEFAX: (248) 539-5055

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 488 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GACAAGGACT CAAAGATGTC ATTGTCCCCG GGACAGTTGT AATGGCAGGC ACAGGTCTTG     60
ATGAACATCA TGTTCTTTTT CATGATCTCG CCATCGGGGC ACTTGAACTC CACCGGCAGT    120
GTGGTGGTTC TGTGCGGTGT GCAGCAGCGG CCGTCCGTGC ACACCCCACA GAACTTAGCC    180
CGGTAGGTCT TCACACTGGT GCAGCCAGAA AGCTCAAACT TGACAGGCTT GGCAATTTTA    240
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CCTGACCATG CAGAGACGAC TCTGCTTCTC CAGCCTGCAG AAGGTATTGT CATTTGGGTA    360
ACCCGGGTTG GAATATGCCC CATCCCACAG GTCTTAAGAA CAGGGCGCTC CACTCTTGTG    420
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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 544 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GCATTCTTGG TTTTGACACT CCTCACTACT GGCTGTGTGA CAACCGCCTG CTGTGCTTGC 120

NAGACCCCAA CAATAAGAGC AATTGGAATG TCTTTAGGGA ATGCTGGAAA CAGGGGCTGN 180

CAGTGATGGT GTCGGGAGTG CATCNTAAAT TAAACACTGA ACTCTGGAAA CCCGAGTCCT 240

TCAGGAAAGA GTTTGGCGAG CAACAAGTAG ACCTAGTCAA TTGTAGGACC AATGAAATCA 300

TCACAGGGAG CCACAGTGGG AGACTTCTGG GATGGATTTG AAGATGTTCC AAACCGTTTG 360

AATGACGACA AAGAAGTCGA ACCANTGGTG TTGAAACTTA AGGACTGGCC GCCATGAGNA 420

AGACTTTAGA AGATNTGATG CCTTCCAGGT TTGATGATCT GATGGCCCCT CATTCCTCTG 480

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TTNT 544

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 642 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AGAGGATTAT CACAAAATTA TCAAACAGCC CATGGACATG GGAACTATCA AGAGGAGACT 240

TGAAAACAAT TACTACTGGG CTGCCTCAGA ATGTATGCAG GATTTTAACA CCATGTTTAC 300

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CCAGTGCCCA TCAGGTGCCT GCTGTCTCTT CTGTGTCGCA TACAGCCCTG TATANCCACC 540

ACCTGAAATA CCTACCACCG TCCTCAACAT TCCCACCCCA TCAGTCATCT CGTCTNCCCT 600

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 619 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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CCCATCATCT TTCCACAGCC AGTGCTTCCT GGTGCTCTCG CAGGGGGAAG GCAATTCGGA 120

AGATGTCGCG GTAGTGTTCC ACGAAGTGAA CCTCCAGGCC CTCTGTGATG AAGGGAGCCA 180

AGTCTGAGAA GTCCTTCCTG TTCTCAGCAG GCAAAATGAT GCAGGTCACT CCCGCACGCT 240

TAGCCGCAAT GGTCTTCTCC TTGATGCCAC CCACAGGCAA CACTTTACCA GTGAGGGAGA 300

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GCAGTGACAA TGGTGCAACC TGCACTAGGG CCATCCTTGG GGGTAGCGCC CTCAGGCACA 420

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GTTCACCTCT AGGCTGCCAT CCTTTGTCCT CCTTGCTGCC ACTAGGCTGG GGCCTCCTTA 600

AAGATGTCTC CCACCAAAC 619

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 607 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACAACTTTAT CGCTGATGTG GTGGAGAAGA TCGCCCCTGC TGTGATTCAC ATTGAACTTT 60

ATCGCAAGCT TCCTTTCTCG AAGAGGGAGG TGCCAGTGGC GAGTGGGTCA GGATTCATTG 120

TGTCGGAGGA TGGACTGATT GTGACAAATG CTCACGTGGT GACCAACAAA AACCGGGTCA 180

AGGTTGGGCT GAAGAATGGA GCGACTTATG AAGCCAAAAT CAAGGATGTG GATGAAAAGG 240

CCGACATTGC GCTTATCAAG ATTGACCACC AGGGTAAGCT GCCAGTCCTG CTGCTTGGCC 300

GCTCCTCCGA GCTGCGGCCA GGAGAATTTG TGGTTGCCAT CGGAACCCCT TCTCTCTTCA 360

AAACACGGTC ACCACTGGGA TCGTCAGCAC CACCCAGCGA GGCGGCAAAG AACTGGGGCT 420

CCGGAACTCC GATATGGACT ACATTCAGAC AGACGCCATC ATCAATTATG GAAACTCCGG 480

AGGCCCGTTA GTAAACCTGG ATGGCGAGGT GATTGGGATT AACACCTTGA AGGTGACGCG 540

GGCATCTCCT TCGCAATTCC ATCCGATAAG ATAAAAAAGT CTTGACANAG TCCCNTGATC 600

GACTGCC 607

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 267 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACCTATCGGC ATCATGTTCC TGATCGGAAG CAAGATTGTG GAAATGCAGG ACCTCATCGT 60

GCTCGTGACT AGCCTCGGGA AATACATCTT CGCCTCTATA CTGGGCCATG TTATCCATGG 120

AGGAATCGTT CTGCCTCTTG TCTATCTTGC TTTTACGAAG AAAAACCCGT TCACGTTCCT 180

CCTGGGCCTC CTCACCCCGT TTGCGACGGC TTTTGCGACC TGTTCTAGCT CAGCAACCCT 240

TCCGTCTATG ATGAATGCAT CGAGGAT 267

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 417 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTATACGACT TCATCGCTGA TGTGGTGGAG AAGATCTCCC CTGCAGTGGT CCACATTACA 60

ACTTTATCGC AAGCTTCCTT TCTCGAACAC GGAGGTGCCA TTGGTCGACT GGGTCAAGAT 120

CCAGTGTGTC GGAGGATGGA CTGATTGTGA CAAATGCTCA CGTNGTGACA CACCAAAATC 180

CGGGTCAGAG TTCAGCTGGG TAATTGAACN ACTCATGAAG CCGGACTCCT CGATGTGGGA 240

TCAAAATGCC CACATTGCGC TTAGCAAAAT TGACCTCCTC GGGTNAACTG CCAGTNCTGC 300

TACGTGGACG CTCCCCCGAG CTCCGGCCAA GAAGAATTTG TGGTTGCCAT CGGGAGACCC 360

CTTCTCTCTT CGGTACCTAC AGTCACCACT GGGGATCGTC TCTCGCCACC CAGAGGA 417

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 528 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TACGTCATTA TATCCACGCC TGAGCTGTCA GAGCTGATCA TCAGCGACCT GGACATGAAC 60

GTGGACCCTG GCACCTACGT GTGCAATGCC ACCAACTCCC AGGGCAGTGC TCGGGAGACC 120

ATCTCACTGC GTGTGCGCAG CCGCCTGGCA GCCCTCTGGC CCTTCCTGGG CATTGTGGCC 180

GAGGTCCTGG TGTTGGTCAC CATCATCTTC ATCTACGAGA AGAGGCGGAA GCCGGACCAG 240

ACCCTGGACG AGGATGATCC TGGCGCCGCC CCACTGAAGG GCAGCGGGTC TCACCTGAAT 300

GACAAGGACA AGAAATGTGC GCCAGAGGGA ACGCCACCTG AAGCGGCGGG GCANGCGGGG 360

AAGGGGAGGT GCCAGGGGCA CTTGACCCCA GCCCATCGTC TGCCTCCACT CCTGTGTCCC 420

ATCCTGTCCC GACCTGAGCC TGCCCAACCC AACTTCTTAT CCCAGCCCAA GTGAAGACAG 480

AGCCTTACTT ACAGAAAACC CATCTGGGAA AAGCAGGCCA CTTGCACT 528

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACAGTGTGAA CATGTGCCCC TCCCTCAAGC AGGGTCAGCA GCGAGCCAAG CATTGTCACA 60

GATGTCTTTC CTGCCCCTCC GGGAGGATTT CGAGTTATAT TCTCAATAAA GACAACTATT 120

TGCTCCGGTG ATAAAAACCT GCAAGAGAGC GTATCAACAG TCCAGGCAGG GGTGAAGTTG 180

GGGTAAGTTG GTTGACCCCA ATCTTGGTGG CACTGAATCT TGAATCCAGA TCCCGGGGGC 240

TAGCTCTCCT ATAANTCCTC TAGGCAAAGC TTCTCACTCT CCAGGAACTC TCCTCCACAA 300

CTCTGNGCCC CNCCGTAANN TCCCATAATN ANNGGACGNC TNGANNCCNC CCTTACATCC 360

CATTTCTTCC ATCCTCCGGN CTCTGNCTNN GACTCCCCCC NGNCTCCCTC CTTC 414

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 266 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACACCGCATC TTCCGCGTCT CGCGGGTCCT CGAACTCAAC TAAGCGAAGG GCGGTCCCCC 60

GCGGCGGTTC TTCAGGTCGA TGTCGCGGAT AGCGCCGTAT TTGTAAAACA CGTCCTCAAT 120

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TGCCGGGCCA CGAATCACAC CACCTCCTGA CATGGCGGCG ACGAAAAGCG CGGACTCGAA 240

AAGAGCCTTC CCACCAAGCC TAGCGT 266

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACGACGTGTG TGCTATTTGC TTGGAGGAAT ATGAAGATGG AGATAAGCTC AGGATCCTTC 60

CCTGTTCCCA TGCTTATCAT TGCAAGTGTG TAGATCCCTG GCTAACTAAA ACCAAAAAGA 120

CCTGTCCAGT CTGCAAACAA AAGGTTGTCC CTTCCCAAGG TGACTCGGAC TCTGACACAG 180

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CAAATACTGT GTGACTGACT TTCAGGTGGT TGGTTTATTT CCCTTAAAAT GTTTATTTAG 480

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GGTATCCAGC CACTCTACTA ACCAGTAACA GACTGGTGCT GTTNNCTCAG GCATCACTTT 660

AGCTCTGGGG ATGAC 675

CLAIMS

What is claimed is:

1. A purified, isolated and cloned nucleic acid polynucleotide having hypoxia regulated activity which has sequences as set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11 or having a complementary or allelic variation sequence thereto or the human homolog thereof.

2. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein encoded by a gene identified by the sequences in the group consisting of SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

3. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a dominant negative peptide directed against at least one of the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ

ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11 or the protein thereof.

4. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

5. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein encoded by a gene identified by the sequences of the group consisting of SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

6. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a dominant negative peptide directed against at least one of the sequences set

forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

7. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

8. A method of providing an apoptotic regulating gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one of the genes identified by the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

9. A method of providing an angiogenesis regulating gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control

sequences operably linked to one of the genes identified by the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

10. A method of regulating response to hypoxia conditions in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

11. A method of providing a hypoxia regulating gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one of the genes identified by the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

12. A method of diagnosing the presence of ischemia in a patient including the steps of analyzing a sample from

the patient for the presence of at least one expressed gene identified by the sequences as set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

13. The method as set forth in claim 12 wherein the sample is a bodily fluid sample and the presence of at least one protein encoded by a gene identified by the sequences set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 or SEQ ID No:11 is ascertained.

14. The method as set forth in claim 12 wherein the sample is a tissue sample and the presence of at least one up-regulated gene identified by the sequences encoded by SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 or SEQ ID No:11 is ascertained.

15. A purified, isolated and cloned nucleic acid polynucleotide having angiogenesis regulated activity which have sequences as set forth in the group comprising SEQ ID SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9,

SEQ ID No:10 and SEQ ID No:11 or having a complementary or allelic variation sequence thereto.

16. A purified, isolated and cloned nucleic acid polynucleotides having apoptosis regulated activity which have sequences as set forth in the group comprising SEQ ID SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11 or having a complementary or allelic variation sequence thereto.

17. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient at least one protein encoded by a gene identified by the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

18. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient at least one protein encoded by a gene identified by the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

19. A method of regulating response to hypoxia conditions in a patient in need of such treatment by administering to a patient at least one protein encoded by a gene identified by the sequences set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5, SEQ ID No:6, SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

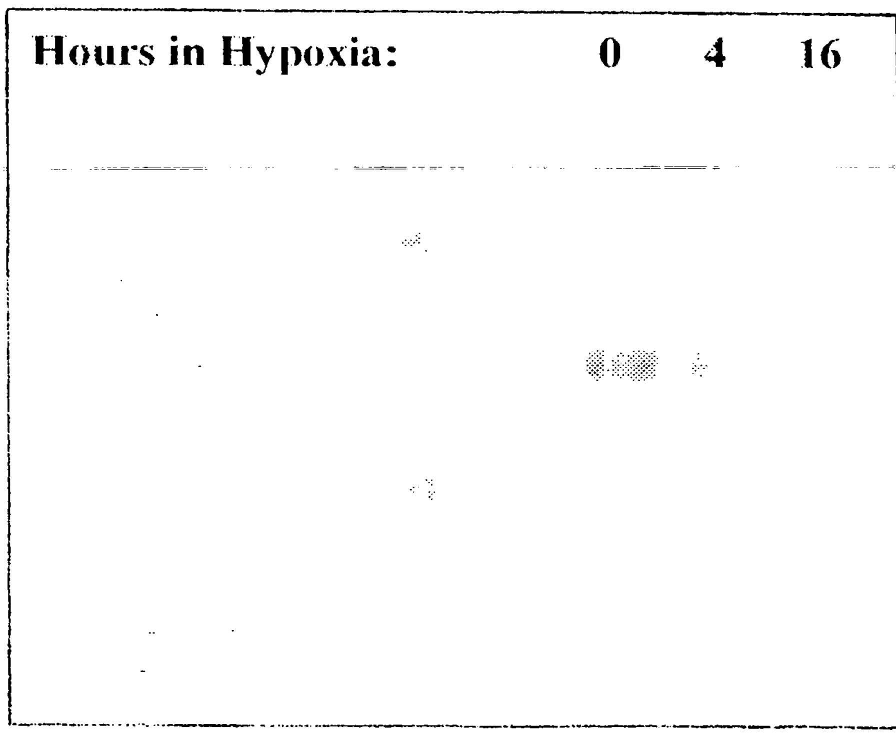

Fig-1

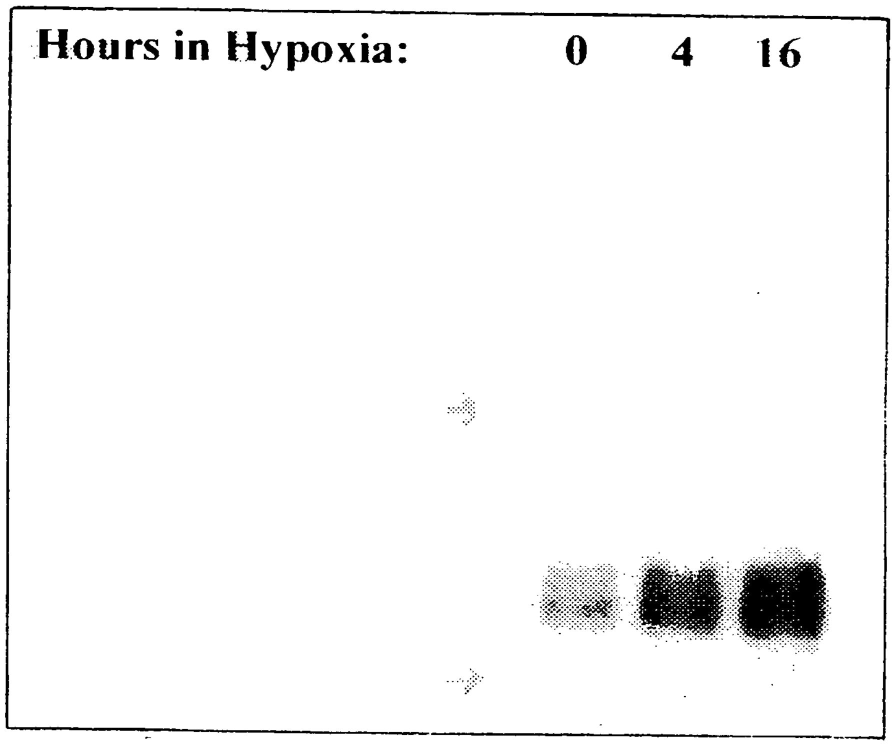

Fig-2

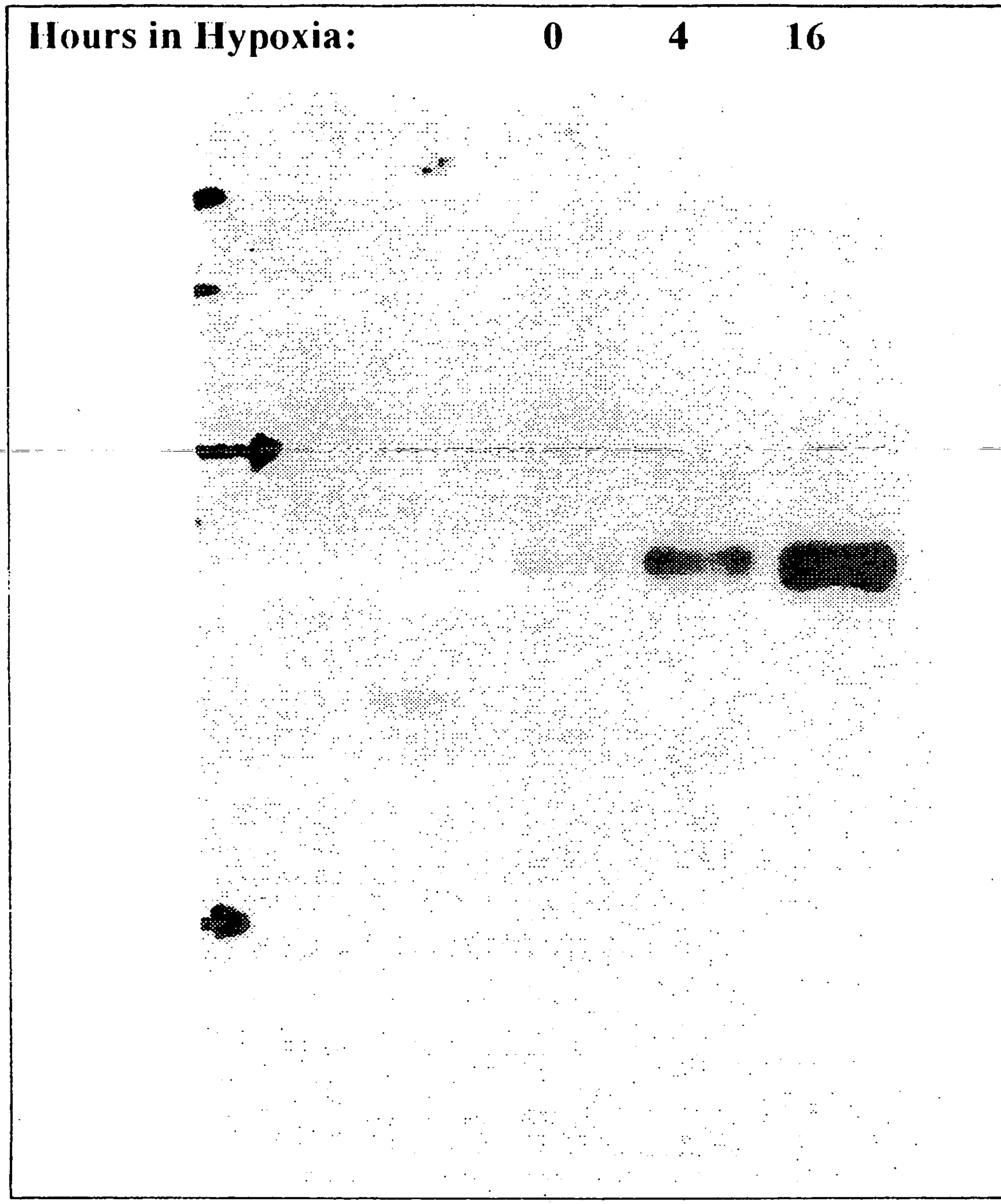

Fig-3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17297

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/02, 04; C12N 15/11; A61K 48/00
US CL :536/ 23.2, 24.5; 435/6, 375; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/ 23.2, 24.5; 435/6, 375; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, EMBASE, CAPLUS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,721,265 A (TRACY et al) 24 February 1998, see column 2.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 22 OCTOBER 1998	Date of mailing of the international search report 30 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SEAN MCGARRY For Telephone No. (703) 308-0196